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## Abstract

The presence of nanoparticles in beverages has raised great concern in terms of potential impacts to consumer health. Herein, carbon dots in beverages kvass, pony malta, pilsner beer, Vivant Storm, and Profit were identified. They were shown to have a strong fluorescence under the excitation of ultraviolet light. The emission peaks shift to longer wavelengths accompanied by a remarkable fluorescence intensity decrease. The carbon dots are in the nanosized range and roughly spherical in appearance. Elemental analysis by X-ray photoelectron spectroscopy demonstrated the composition of Kvass carbon dots to be C 83.17%, O 13.83%, and N 3.00%. No cytotoxicity was found at concentrations up to 20 mg/mL for human tongue squamous carcinoma cells, and they can be directly applied in both carcinoma and onion epidermal cell imaging. This work represents the first report of the carbon dots present in beverages, providing valuable insights into these nanoparticles for future biological imaging.

## Keywords

Beverage, Carbon dots, Cytotoxicity, Fluorescence bioimaging

## Disciplines

Agriculture | Bioresource and Agricultural Engineering | Food Chemistry

## Comments

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**ABSTRACT:** The presence of nanoparticles in beverages has raised great concern in terms of potential impacts to consumer health. Herein, carbon dots in beverages kvass, pony malta, pilsner beer, Vivant Storm, and Profit were identified. They were shown to have a strong fluorescence under the excitation of ultraviolet light. The emission peaks shift to longer wavelengths accompanied by a remarkable fluorescence intensity decrease. The carbon dots are in the nanosized range and roughly spherical in appearance. Elemental analysis by X-ray photoelectron spectroscopy demonstrated the composition of Kvass carbon dots to be C 83.17%, O 13.83%, and N 3.00%. No cytotoxicity was found at concentrations up to 20 mg/mL for human tongue squamous carcinoma cells, and they can be directly applied in both carcinoma and onion epidermal cell imaging. This work represents the first report of the carbon dots present in beverages, providing valuable insights into these nanoparticles for future biological imaging.

**KEYWORDS:** carbon dots, beverage, cytotoxicity, fluorescence bioimaging

## INTRODUCTION

The presence of nanoparticles (NPs) in foods and beverages has raised considerable debate because of potential health concerns.<sup>1</sup> Some foods sold in supermarkets worldwide contain NPs that were either produced during the manufacturing process or added artificially to enhance flavor, to improve supplement delivery, to extend shelf life, and/or to generate brighter, more attractive colors. Because public perception of food containing NPs is usually negative, and in the absence of mandatory product labeling on NPs anywhere in the world, it is difficult for the general public to obtain information on whether or not certain commercial foods contain NPs. Food manufacturers either are not interested in providing this information about their NP-containing foods or may not be aware that their food contains NPs. Additionally, our knowledge about the presence of NPs in food is still in its infancy, and little is known about to what extent NPs are present in foods.

Recently, there has been an increasing number of works reporting the presence of NPs in human foods. For example, Sk et al.<sup>2</sup> reported the presence of carbon NPs in different carbohydrate-based food caramels (e.g., bread, jaggery, sugar caramel, corn flakes, and biscuits) where the preparation process involves heating of the starting material. The amorphous carbon NPs were spherical in shape with a size range of 4–30 nm and exhibited excitation tunable fluorescence activity. Blasco et al.<sup>3</sup> discussed the state of the art in identifying and determining NPs in foods, including the determination of the particle size distribution, the chemical composition, and physical and chemical properties of nanomaterials. They also

addressed the need for legislative consideration to disseminate information on how nanomaterials are determined in foods. Increasingly, food products have been found to contain some sort of nanomaterials. Weir et al.<sup>4</sup> reported that titanium dioxide (TiO<sub>2</sub>) is present in many foods, personal care products, and other consumer goods. They quantitated the amount of TiO<sub>2</sub> in common food products and discussed the environmental impact of the nanoscale fraction of TiO<sub>2</sub>. Kammer et al.<sup>5</sup> reported the separation and characterization of NPs in complex food and environmental samples by field-flow fractionation (FFF). In a complex or heterogeneous food sample, each step of this sequence is an individual challenge, and FFF is shown to be one of the most promising techniques. We recently reported the presence of carbon dots in commercial Nescafé instant coffee.<sup>6</sup> TEM analysis revealed that the extracted carbon dots had an average size of about 4.4 nm. They were well-dispersed in water and strongly fluorescent under excitation light with a quantum yield about 5.5%. These carbon dots have been directly applied in cellular and FISH imaging, suggesting that the nanomaterials present in commercial coffee product may have more biological applications such as image-guided drug delivery.

Limitation in our knowledge is partly due to a lack of proper methodology for the detection and characterization of nanomaterials in complex matrices (i.e., water, soil, or foods).

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If NPs are not emitting any signal when mixed within food samples, it is difficult to find them. However, some NPs present in food samples, such as carbon dots, could emit fluorescent signals; hence, they can be extracted easily by using size exclusion chromatography. Carbon dots belong to a new class of quantum dot-like NPs that have wavelength-tunable emission properties, good photostability, and biocompatibility.<sup>7–13</sup> They are considered promising candidates for potential biomedical applications because they are safer and nontoxic both *in vitro* and *in vivo*.<sup>7,8,14–17</sup> The preparation, physicochemical properties, and applications of carbon dots in metal ion sensing, cell imaging, and photochemical catalysis have been intensively documented worldwide since the original report in 2004.<sup>15,18–20</sup> We have discovered that the fluorescence of Nescafé Original instant coffee originated from the presence of carbon dots,<sup>6</sup> which has motivated us to analyze the presence of carbon dots in other common beverages.

Carbon dots in beverages are being consumed constantly with no known toxicity. Thus, it is reasonable to believe that they may be safely utilized in many biological applications. In this study, the presence of carbon dots in common beverages including fermented beverages (kvass, pony malta, pilsner beer) and sport beverages (Vivant and Profit) were investigated. The extraction methods of carbon dots were established and their fluorescence properties were characterized. The representative carbon dots from kvass were systematically studied, including their surface chemistry, fluorescence lifetime, stability, and cytotoxicity. Their application as bioimaging agents in tumor cells and onion epidermal cells was also explored.

## MATERIALS AND METHODS

**Materials.** Kvass was purchased from Harbin Tyurin in Dawes Food Co., Ltd. (Harbin, China). Pony malta was purchased from Cervecería Nacional Cn S.A. Co. (Guayaquil, Ecuador). Pilsner beer was also purchased from Cervecería Nacional Cn S.A. Co. Vivant Storm was purchased from Corporation Azende S.A. Co. (Guayaquil, Ecuador). Profit was purchased from Industrias Lácteas Toni S.A. (Guayaquil, Ecuador). Quinine sulfate was purchased from Aladdin Reagent Co. (Shanghai, China). Sephadex gel G-25 was purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Aladdin Reagents Co., Ltd. (Shanghai, China). Chinese hamster ovary (CHO) cell line was a gift kindly provided by the Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences. RPMI-1640 medium was purchased from HyClone Laboratories (Logan, UT, USA). Onion was purchased from the local market. Human tongue squamous carcinoma cell line Tca-8113 was purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China).

**Instrumentation.** Absorption spectra were recorded at room temperature on a UV-2550 UV-vis spectrophotometer (Shimadzu, Japan). The fluorescence spectra were measured by an LS55 fluorescence spectrometer (PerkinElmer, USA). The steady-state fluorescence and time-resolved fluorescence decays were recorded using a FluoroMax-4 spectrofluorometer (Horiba Scientific Co., Albany, NY, USA) with a 376 nm laser as the excitation source. Transmission electron microscopy (TEM) measurements were performed on a model JEM-2000EX transmission electron microscope (JEOL, Akishima, Japan) for characterization of the shape and size of the carbon dots by deposition on 400-mesh C-coated Cu grids. X-ray photoelectron spectroscopy (XPS) spectra were used to characterize the chemical composition using an Escalab 250 Xi X-ray photoelectron spectrometer (Thermo Scientific, USA). The Fourier transform infrared spectroscopy (FTIR) spectra were measured by VECTOR

22 (Bruker, Germany) with the KBr pellet technique ranging from 500 to 4000 cm<sup>-1</sup>. Fluorescent microscope imaging was conducted with a laser-based point scanning FV1000 confocal fluorescent microscope (Olympus, Japan), equipped with a 40× objective lens in *xy* mode with 600 × 600 pixel resolution; 405 and 488 nm lasers were used for excitation of carbon dots with emitted light collected between 425 and 475 nm and between 500 and 600 nm, respectively.

**Carbon Dots Extraction.** Beverages were concentrated and purified. For kvass, 150 mL of sample was added to a beaker and stirred at room temperature for 4 h to remove the gas. The kvass was then lyophilized. To further purify the kvass, 1 mL of the concentrated aqueous solution was added to a Sephadex G-25 column and the fluorescent fractions were collected. Other soluble fractions were retained on the column and eluted after the C-dots were collected. The resulting fluorescent carbon dot fractions (~20 mL) were further lyophilized and stored in the dark at 4 °C for further characterization and use.

**Fluorescence Quantum Yield and Lifetime Measurement.** To calculate fluorescence quantum yield ( $\Phi$ ), eq 1 was used

$$\Phi_1 = \Phi_2 I_1 A_2 \eta_1^2 / I_2 A_1 \eta_2^2 \quad (1)$$

where  $I_1$  and  $I_2$  are fluorescence intensities of carbon dots and the quinine sulfate standard, respectively;  $A_1$  and  $A_2$  are optical densities of carbon dots and the standard, respectively;  $\eta_1$  and  $\eta_2$  are refractive indices of carbon dots and the standard, respectively. The standard quantum yield of  $\Phi_2$  was 0.54 for quinine sulfate at 360 nm in 0.1 M H<sub>2</sub>SO<sub>4</sub> (refractive index:  $\eta_2 = 1.2$ ), and carbon dots were dissolved in water (refractive index:  $\eta_1 = 1.2$ ). Absorbance values of each solution in cuvette were maintained under 0.1 at the excitation wavelength to minimize reabsorption effects. Excitation and emission slit widths were both 4.5 nm for the fluorescence spectra measurement. The fluorescence lifetime ( $\tau$ ) of the C-dots was calculated using eq 2<sup>7</sup>

$$\tau = (A_1 \tau_1 + A_2 \tau_2 + A_3 \tau_3) / (A_1 + A_2 + A_3) \quad (2)$$

where  $A_i$  is the fractional contributions of time-resolved decay lifetime of  $\tau_i$ . The average fluorescence lifetime of carbon dots from kvass was calculated to be 10.59 ns.

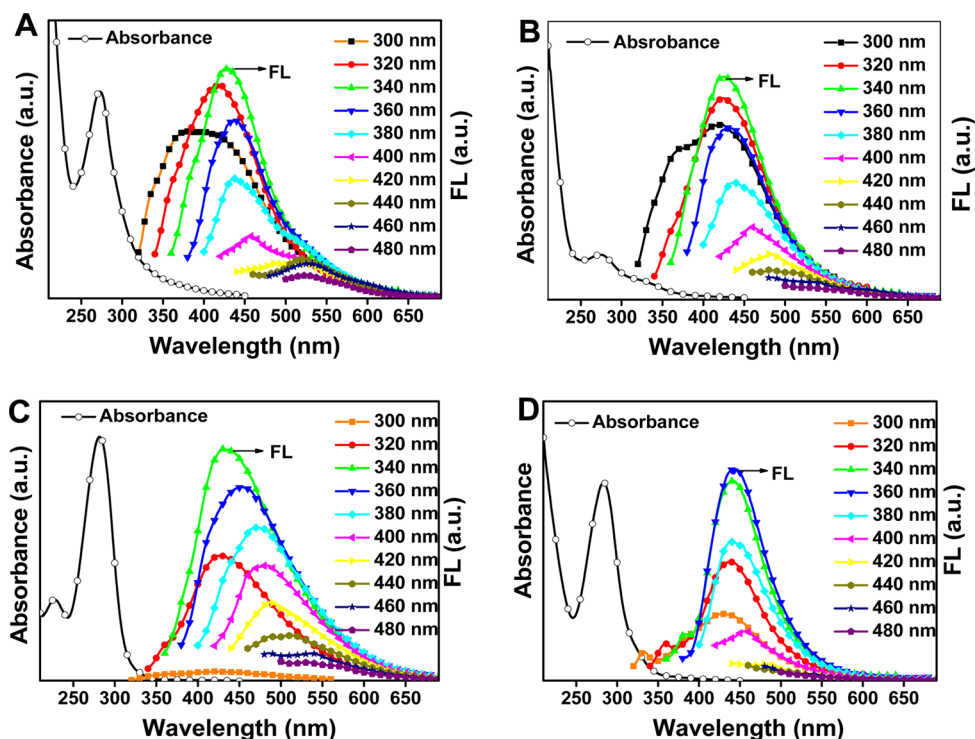
**pH Effect on Fluorescence Intensity.** Various pH (pH 2–11) solutions were prepared by adding different amounts of 0.2 mmol/L NaOH solution to the Britton–Robinson buffer consisting of 0.04 M H<sub>3</sub>BO<sub>3</sub>, 0.04 M H<sub>3</sub>PO<sub>4</sub>, and 0.04 M CH<sub>3</sub>COOH. To 2 mL of Britton–Robinson buffer solution was added 200  $\mu$ L of carbon dots solution (1 mg/mL), and the fluorescence intensity was recorded with a fluorescence spectrometer using a 330 nm excitation wavelength and a 1200 nm/min scan rate. Measurements were done in triplicate.

**Cytotoxicity Assay.** CHO cells were seeded in 96-well plates at a density of  $5 \times 10^3$  cells/well and cultured for 24 h in 10% serum containing DMEM (Sigma-Aldrich Co. LLC) supplemented with penicillin (100 units/mL) and streptomycin (100  $\mu$ g/mL) in a humidified atmosphere with 5% CO<sub>2</sub> at 37 °C. After incubation with different concentrations (0, 0.31, 0.63, 1.25, 2.5, 5.0, 10.0, 20.0, and 40.0 mg/mL,  $n = 3$ ) of carbon dots from kvass for 24 h, 20  $\mu$ L of MTT (5 mg/mL) reagent was added to each cell well. After further incubation for 3 h, the cells were washed with PBS three times, and 100  $\mu$ L of dimethyl sulfoxide (DMSO) was added. The optical density (OD) of the mixture was measured at 570 nm using a microplate reader. The cell viability was estimated according to eq 3

$$\text{cell viability (\%)} = (\text{OD}_{\text{treated}} / \text{OD}_{\text{control}}) \times 100\% \quad (3)$$

where OD<sub>control</sub> is measured in the absence of agent and OD<sub>treated</sub> is the intensity of carbon dots from kvass. The viability was expressed as the percentages of viable cells compared to the survival of a control group (untreated cells as controls of 100% viability).

**In Vitro Tumor Cell Imaging.** Human tongue squamous carcinoma (Tca)-8113 cells were cultured and maintained in DMEM containing 10% FBS, antibiotic–antimycotic solution L-glutamine (2 mmol/L), and nonessential amino acids (1%) in 5% CO<sub>2</sub> at 37 °C. After the cells were trypsinized and seeded in tissue culture plates at an initial cell density of  $1 \times 10^5$  cells/well, they were



**Figure 1.** UV-vis absorption and fluorescence (FL) spectra of (A) pony Malta, (B) pilsner beer, (C) Vivant, and (D) Profit.

treated with the carbon dots from kvass at a concentration of 10 mg/mL. After incubation at 37 °C for 24 h, the Tca-8113 cells were washed three times with fresh media and imaged with a laser confocal fluorescent microscope with excitation of 405 and 488 nm laser and emission of 425–475 and 500–600 nm for blue and green region imaging, respectively.

**Onion Epidermal Cells Imaging.** A small piece of the epidermal membrane of a fresh onion was laid flat on the surface of a slide, and 0.9% saline containing 40 mg/mL kvass carbon dots was added. The sample was then covered with a thin glass coverslip ensuring that there were no air bubbles. After 6 h, onion epidermal cells were imaged with a laser confocal fluorescent microscope with excitation of a 405 nm laser, and the collecting wavelengths were 425–475 nm for blue emission.

## RESULTS AND DISCUSSION

**Extraction of Carbon Dots and Their Fluorescent Properties.** The beverages, such as fermented beverages (kvass, pony Malta, pilsner beer) and sport beverages (Vivant Storm and Profit), are all common drinks. Carbon dots were extracted from all five beverages by using a size exclusion Sephadex G-25 column. The extraction of carbon dots from the five commercial beverages can be simply done by column chromatographic separation. Sephadex G-25 was used here because it was a well-established gel filtration medium prepared by cross-linking dextran with epichlorohydrin for desalting. The overall production yield of the carbon dots is about 2–5% w/w calculated from the beverages. Similar results were also reported as 2–3% w/w of carbon dots present in bread, jaggery, corn flakes, and biscuit.<sup>2</sup> The aqueous solution of carbon dots from the five beverages exhibited strong fluorescence under excitation of ultraviolet light at 365 nm. The UV-vis absorption and fluorescence spectra of the aqueous solution of carbon dots are shown in Figure 1. A strong absorption feature centered at 275 nm in the UV-vis absorption spectra was observed for all of the carbon dots

derived from the four beverages, indicating the presence of aromatic  $\pi$  orbitals. When excited in the range of 300–480 nm, these carbon dots emit fluorescence around 430–450 nm. The emission peaks shift to longer wavelengths accompanied by a remarkable fluorescence intensity decrease. The bathochromic emission phenomenon is consistent with previous reports on carbon dots.<sup>16</sup> Their fluorescent properties are summarized in Table 1. The fluorescent quantum yields of C-dots from the

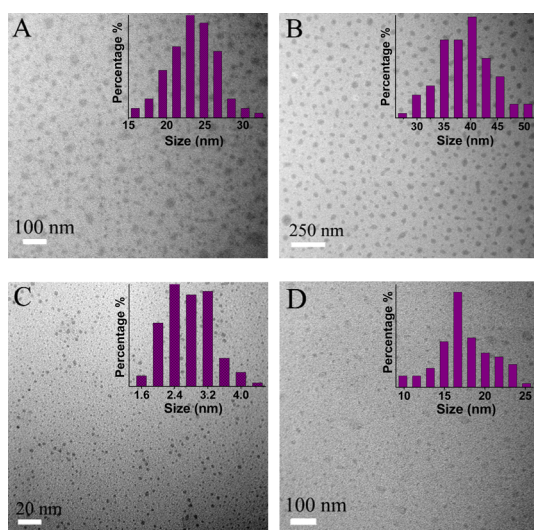
**Table 1.** Fluorescent Properties of the Carbon Dots from Beverages

C-dots	av size (nm)	max ex (nm)	max em (nm)	fwhm (nm)	quantum yield (%)
pony Malta	23.5	352	447	100	6.10
pilsner	39.1	329	414	101	1.48
Vivant Storm	2.8	326	399	107	1.90
Profit	17.5	352	447	80	11.90
kvass	5.0	328	411	106	2.22

five beverages are in the range of 1.48–11.9%. The morphology of carbon dots from pony Malta, pilsner beer, Vivant Storm, and Profit was characterized using TEM by dropping a dilute aqueous solution of the carbon dots on a carbon-coated copper grid. The carbon dots are nearly spherical in shape and well dispersed, with average sizes of 23.5, 39.1, 2.8, and 17.5 nm for pony Malta, pilsner beer, Vivant Storm, and Profit, respectively (Figure 2; Table 1). This indicates the presence of nanoparticles in the four beverages. An organic macromolecule that has absorbed a small fluorescence molecule might not be visible in the classic TEM without counterstaining, due to the low contrast of the light elements such as carbon and hydrogen.

Pony Malta is a nonalcoholic malt beverage. Currently the drink is produced and marketed in several countries in South and Central America, including Colombia, Peru, Ecuador,





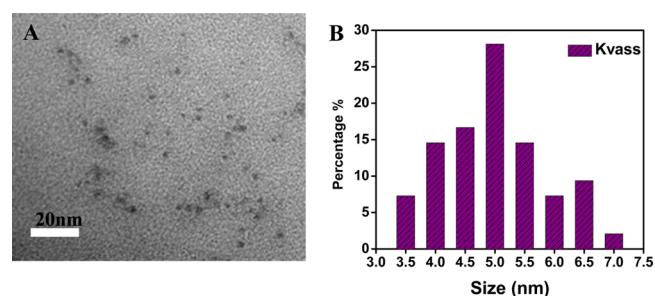
**Figure 2.** TEM images of (A) pony malta, (B) pilsner beer, (C) Vivant, and (D) Profit carbon dots. (Insets) Corresponding particle size histograms.

Panama, and Venezuela. The beverage is made from barley malt, sugar, fiber, protein, niacin, thiamin, riboflavin, and calcium. The origin of carbon dots from pony malta possibly is due to the heating of the starting materials, such as sugar, which has been reported to form carbon dots in food products.<sup>2</sup> Pilsner beer is an alcoholic beverage produced by the saccharification of starch and the subsequent fermentation of the resulting sugar. The basic ingredients of pilsner beer are water, malt, hops, and yeast. The preparation of beer is called brewing. Any starting materials involved in the heating process could produce the carbon dots. Sugar is a common ingredient in the other two beverages, Vivant Storm and Profit, as well. We believe the origin of carbon dots in all four beverages is due to the heating of sugar during the manufacturing process.

**Characterization of Carbon Dots from Kvass.** The carbon dots derived from kvass were selected as representative nanoparticles, and their properties were investigated thoroughly. Kvass is a fermented beverage made from black or regular rye bread and is classified as a nonalcoholic drink by Russian and Ukrainian standards, as the alcohol content from fermentation is typically <1.2%. Carbon dots have been found in bread, where the baking process involves heating of the starting materials.<sup>2</sup> Because carbon dots are highly water-soluble, they might be dissolved in the beverage kvass during

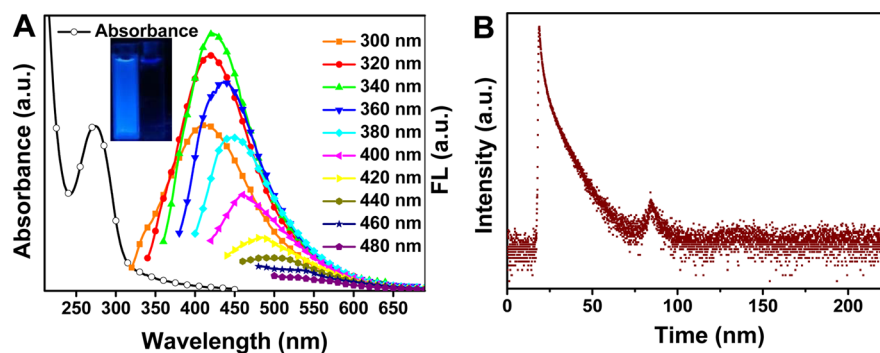
the fermentation process. Therefore, the origin of carbon dots from kvass could be attributed to the bread. The kvass carbon dots were analyzed by UV–vis and fluorescence spectroscopy, respectively. **Figure 3A** shows the typical absorption and fluorescence emission spectra measured from the kvass carbon dots in aqueous solution. The absorption spectrum exhibits only one peak around 265 nm, which extends to 500 nm without noticeable fine structures. Similar to previous studies, the peak at 265 nm denotes  $\pi-\pi^*$  transition of aromatic C=C bonds.<sup>21</sup> The fluorescence emission spectrum displays an emission maximum at 425 nm for excitation at 360 nm, and the emission wavelength and intensity show clear excitation wavelength dependency, similar to that of previously reported carbon dots.<sup>22</sup> This complex behavior might be associated with a variety of emitting centers present in the carbon dots suspension.<sup>23</sup> It clearly indicates the presence of carbon dots in kvass. The inset in **Figure 3A** shows the optical image of kvass carbon dots in aqueous solution irradiated by a 365 nm UV lamp, and the fluorescence was strong enough to be seen with the naked eye. A typical luminescence lifetime ( $\tau$ ) was also measured (**Figure 3B**), where a value of 10.59 ns was obtained. The decay of fluorescence emission was non-monoexponential for the kvass carbon dots suspension. The possible mechanism of fluorescence might be attributed to the radiative recombination of the energy-trapping sites on the carbon dots.<sup>6,18</sup> Using quinine bisulfate (QY = 0.54 in 0.1 M H<sub>2</sub>SO<sub>4</sub>) as reference, the QY of the kvass carbon dots in aqueous solution was determined to be 2.22%, comparable to previous results.<sup>2</sup>

The TEM image (**Figure 4A**) illustrates that the kvass carbon dots are spherical in shape and well dispersed, with an average



**Figure 4.** (A) Representative TEM image of kvass carbon dots and (B) particle size histogram.

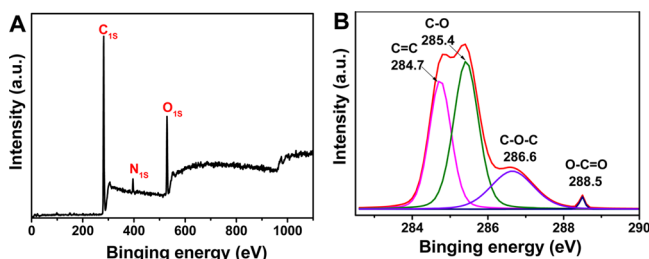
size of  $5.0 \pm 0.7$  nm, in good agreement with sizes of carbon dots observed previously.<sup>6</sup> The TEM result further confirmed



**Figure 3.** (A) UV–vis absorption and fluorescence (FL) spectra and (B) fluorescence decay curve of kvass carbon dots. (Inset) Photograph of the kvass carbon dot aqueous solution and water under the excitation of UV light.

the presence of carbon dots in the kvass beverage. The size distribution of the carbon dots was relatively narrow, with the majority falling within 3.5–7.0 nm based on the statistical analysis of 100 particles (Figure 4B). The ultrasmall size of the kvass carbon dots may facilitate their entry into live cells for bioimaging applications.

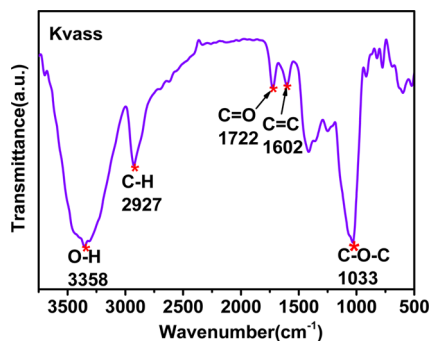
XPS elemental analysis revealed the composition of the kvass carbon dots to be C 83.17%, O 13.83%, and N 3.00%, thus indicating these dots are carbon-rich nanodots. Figure 5A



**Figure 5.** (A) XPS spectrum and (B) high-resolution XPS spectra of C 1s of kvass carbon dots.

shows the XPS spectrum of kvass carbon dots, in which three peaks were observed at 285, 532, and 399.4 eV, corresponding to carbon, oxygen, and nitrogen, respectively. Figure 5B shows the high-resolution C 1s peak of the carbon dots, which could be fitted into four peaks at 284.7, 285.4, 286.6, and 288.5 eV. They were attributed to C=C, C—O, C—O—C, and O—C=O bonds, respectively.<sup>21</sup> These data show that the kvass carbon dots are mainly composed of graphitic carbon (sp<sup>2</sup>) and carbon defects (sp<sup>3</sup>), and abundant hydroxy and carbonyl/carboxylate groups are found at their surfaces.<sup>24</sup>

FTIR spectroscopy was used to gain further structural insights about the kvass carbon dots. As shown in Figure 6, a



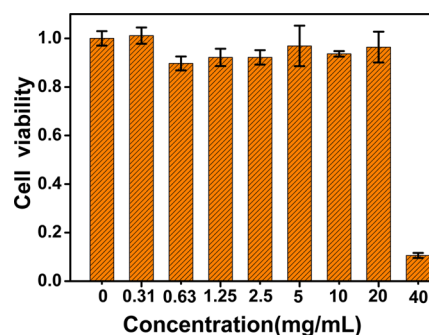
**Figure 6.** FTIR spectra of kvass carbon dots.

broad and intense peak around 3358 cm<sup>-1</sup> was attributed to the O—H stretching vibration, indicating the existence of large numbers of hydroxyl groups. The peak at 2927 cm<sup>-1</sup> corresponds to the C—H vibrations of methylene. The predominant peak of a C=O group at 1722 cm<sup>-1</sup> and C=C double bonds at 1602 cm<sup>-1</sup> can also be observed, consistent with the XPS results. Another strong peak with high intensity at 1033 cm<sup>-1</sup> indicates the existence of aromatic alkoxy bonds in the kvass carbon dots. These results revealed that there are many carboxyl/hydroxyl groups on the surface of the kvass carbon dots. These hydrophilic groups greatly improve the aqueous dispersibility and stability of the carbon dots in

aqueous solution and may facilitate their applications in biomedical fields.<sup>21</sup>

Interestingly, the fluorescence intensity of the kvass carbon dots appeared to be pH independent with the pH ranging from 2 to 11. This is quite similar to the carbon dots extracted from Nescafe Original instant coffee,<sup>6</sup> whereas the carbon dots from candle soot displayed a pH-dependent fluorescence intensity.<sup>25</sup> These results indicated that the radiative recombination of the energy-trapping sites on the kvass carbon dots was not affected by changing pH from alkaline to acidic. Moreover, the effect of sodium chloride concentration on fluorescence intensity of the kvass carbon dots was also investigated. When the sodium chloride concentration increased from 0 to 2.5 mol/L, the fluorescence intensity decreased only 20%. The good stability of the kvass carbon dots in a wide range of pH and NaCl concentration suggested that these carbon dots might find good biological applications.

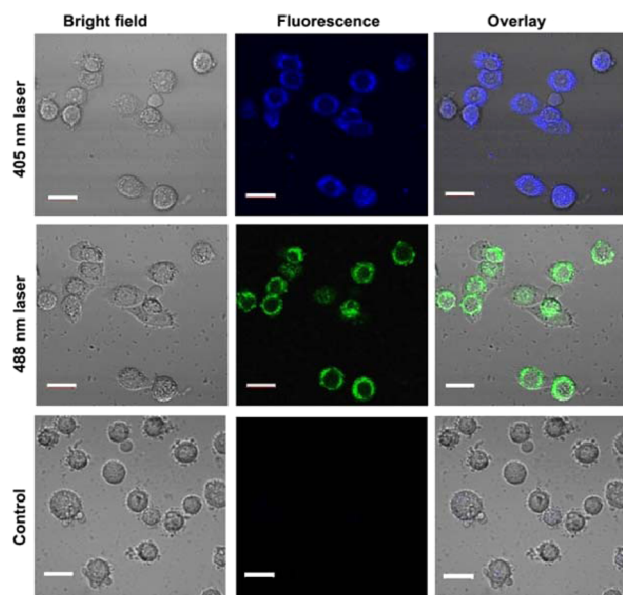
**Cytotoxicity Study of the Kvass Carbon Dots.** The cytotoxicity of the kvass carbon dots was assessed by MTT assay with CHO cells. Figure 7 shows the cell viability profile



**Figure 7.** Cytotoxicity of kvass carbon dots with increasing concentrations from 0 to 40 mg/mL in CHO cells. All values are the average of triplicate measurements.

after incubation with the kvass carbon dots at concentrations of 0.31–40 mg/mL for 24 h. The results suggested that the cytotoxicity of the kvass carbon dots was negligible up to a concentration of 20 mg/mL, whereas most of synthesized carbon dots are toxic at 2 mg/mL.<sup>15,17,26</sup> However, the cell viability fell significantly when the concentration of the kvass carbon dots became >20 mg/mL. Overall, the cytotoxicity of the kvass carbon dots was only marginal even at relatively high concentration (20 mg/mL), which makes them suitable for bioimaging applications. Moreover, further experiments are required to study their safety issue for the new carbon nanomaterials in future biomedical applications, although carbon is not considered an intrinsically toxic element.

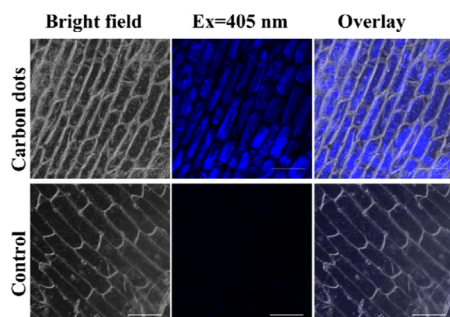
**In Vitro Tumor Cell Imaging.** To illustrate the bioimaging potential of the kvass carbon dots, we studied their in vitro uptake by human tongue squamous carcinoma (Tca-8113) cells. Cells were cultured and maintained in DMEM containing the kvass carbon dots for 24 h. Figure 8 shows confocal images of Tca-8113 cells treated with the kvass carbon dots at bright field; excitation wavelengths were 488 and 405 nm, respectively. The human tongue squamous carcinoma cells incubated with the kvass carbon dots became bright at  $\lambda_{ex}$  405 and 488 nm, as compared to the control cells without the addition of the carbon dots. The fluorescent images of Tca-8113 cells clearly show the agglomeration of blue and green fluorescent carbon dots distributed inside the cytoplasm area of the tumor cells.



**Figure 8.** Laser scanning confocal microscopy images of Tca-8113 cells under bright field, by excitation at 488 and 405 nm. Cells without carbon dots were used as a control. Scale bar = 30  $\mu\text{m}$ .

Notably, the excitation-dependent fluorescence of the kvass carbon dots made it possible for multicolor (blue and green) cell imaging.

**Onion Epidermal Cell Imaging.** The kvass carbon dots were also used for imaging onion epidermal cells inside the onion skin. The kvass carbon dots were directly applied to the onion skin. After 6 h, the onion epidermal cells were imaged at bright field, with an excitation wavelength of 405 nm. As shown in Figure 9, strong fluorescence of the kvass carbon dots was



**Figure 9.** Laser scanning confocal microscopy images of onion epidermal cells under bright field, by excitation at 405 nm. Cells without carbon dots were used as a control. Scale bar = 200  $\mu\text{m}$ .

observed as compared to the control, indicating that the fluorescence was from the carbon dots. It can be clearly seen that the kvass carbon dots were uniformly distributed in the cytoplasm area, emitting strong blue fluorescence. The carbon dots obtained from kvass can serve as a potential substitute for organic dyes or semiconductor quantum dots in bioimaging.

In the present study, the fluorescent carbon dots were extracted from five beverages, kvass, pony malta, pilsner beer, Vivant, and Profit. TEM analysis demonstrated that the extracted carbon dots are in the nanosize range. Carbon dots from the five beverages exhibited an excitation-dependent emission behavior. XPS elemental analysis revealed the kvass carbon dots are carbon-rich nanodots with excellent pH and

ion strength stability and excellent biocompatibility. The kvass carbon dots can be directly applied in the imaging of human tongue squamous carcinoma and onion epidermal cells. The discovery of the NPs in human beverages may provide valuable insights into their safety, distribution, and potential biological imaging applications.

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### Notes

The authors declare no competing financial interest.

## REFERENCES

- (1) Institute of Medicine (US) Food Forum. *Nanotechnology in Food Products: Workshop Summary. Safety and Efficacy of Nanomaterials in Food Products*; National Academy Press: Washington, DC, USA, 2009.
- (2) Sk, M. P.; Jaiswal, A.; Paul, A.; Ghosh, S. S.; Chattopadhyay, A. Presence of amorphous carbon nanoparticles in food caramels. *Sci. Rep.* **2012**, 2, DOI: 10.1038/srep00383.
- (3) Blasco, C.; Picó, Y. *Nanoparticles in Foods, Determination of*; Wiley: Hoboken, NJ, USA, 2013.
- (4) Weir, A.; Westerhoff, P.; Fabricius, L.; Hristovski, K.; von Goetz, N. Titanium dioxide nanoparticles in food and personal care products. *Environ. Sci. Technol.* **2012**, 46, 2242–2250.
- (5) von der Kammer, F.; Legros, S.; Larsen, E. H.; Loeschner, K.; Hofmann, T. Separation and characterization of nanoparticles in complex food and environmental samples by field-flow fractionation. *TrAC, Trends Anal. Chem.* **2011**, 30, 425–436.
- (6) Jiang, C.; Wu, H.; Song, X.; Ma, X.; Wang, J.; Tan, M. Presence of photoluminescent carbon dots in Nescafe original instant coffee: applications to bioimaging. *Talanta* **2014**, 127, 68–74.
- (7) Yan, H.; Tan, M.; Zhang, D.; Cheng, F.; Wu, H.; Fan, M.; Ma, X.; Wang, J. Development of multicolor carbon nanoparticles for cell imaging. *Talanta* **2013**, 108, 59–65.
- (8) Feng, L.; Yang, X.; Shi, X.; Tan, X.; Peng, R.; Wang, J.; Liu, Z. Polyethylene glycol and polyethylenimine dual-functionalized nanographene oxide for photothermally enhanced gene delivery. *Small* **2013**, 9, 1989–1997.
- (9) Liu, Z. Y.; Zhang, C. L.; Luo, L.; Chang, Z.; Sun, X. M. One-pot synthesis and catalyst support application of mesoporous N-doped carbonaceous materials. *J. Mater. Chem.* **2012**, 22, 12149–12154.
- (10) Mitra, S.; Chandra, S.; Kundu, T.; Banerjee, R.; Pramanik, P.; Goswami, A. Rapid microwave synthesis of fluorescent hydrophobic carbon dots. *RSC Adv.* **2012**, 2, 12129–12131.
- (11) Zhang, Y. Q.; Ma, D. K.; Zhuang, Y.; Zhang, X.; Chen, W.; Hong, L. L.; Yan, Q. X.; Yu, K.; Huang, S. M. One-pot synthesis of N-doped carbon dots with tunable luminescence properties. *J. Mater. Chem.* **2012**, 22, 16714–16718.
- (12) Zhao, Q. L.; Zhang, Z. L.; Huang, B. H.; Peng, J.; Zhang, M.; Pang, D. W. Facile preparation of low cytotoxicity fluorescent carbon nanocrystals by electrooxidation of graphite. *Chem. Commun.* **2008**, 5116–5118.
- (13) Wang, B. B.; Wang, Y. F.; Wu, H.; Song, X. J.; Guo, X.; Zhang, D. M.; Ma, X. J.; Tan, M. Q. A mitochondria-targeted fluorescent probe based on TPP-conjugated carbon dots for both one- and two-photon fluorescence cell imaging. *RSC Adv.* **2014**, 4, 49960–49963.



- (14) Qiao, Z. A.; Wang, Y. F.; Gao, Y.; Li, H. W.; Dai, T. Y.; Liu, Y. L.; Huo, Q. S. Commercially activated carbon as the source for producing multicolor photoluminescent carbon dots by chemical oxidation. *Chem. Commun.* **2009**, 46, 8812–8814.
- (15) Salinas-Castillo, A.; Ariza-Avidad, M.; Pritz, C.; Camprubi-Robles, M.; Fernandez, B.; Ruedas-Rama, M. J.; Megia-Fernandez, A.; Lapresta-Fernandez, A.; Santoyo-Gonzalez, F.; Schrott-Fischer, A.; Capitan-Vallvey, L. F. Carbon dots for copper detection with down and upconversion fluorescent properties as excitation sources. *Chem. Commun.* **2013**, 49, 1103–1105.
- (16) Tan, M.; Zhang, L.; Tang, R.; Song, X.; Li, Y.; Wu, H.; Wang, Y.; Lv, G.; Liu, W.; Ma, X. Enhanced photoluminescence and characterization of multicolor carbon dots using plant soot as a carbon source. *Talanta* **2013**, 115, 950–956.
- (17) Baker, S. N.; Baker, G. A. Luminescent carbon nanodots: emergent nanolights. *Angew. Chem., Int. Ed.* **2010**, 49, 6726–6744.
- (18) Sun, Y. P.; Zhou, B.; Lin, Y.; Wang, W.; Fernando, K. A. S.; Pathak, P.; Meziani, M. J.; Harruff, B. A.; Wang, X.; Wang, H. F.; Luo, P. J. G.; Yang, H.; Kose, M. E.; Chen, B. L.; Veca, L. M.; Xie, S. Y. Quantum-sized carbon dots for bright and colorful photoluminescence. *J. Am. Chem. Soc.* **2006**, 128, 7756–7757.
- (19) Chen, P. C.; Chen, Y. N.; Hsu, P. C.; Shih, C. C.; Chang, H. T. Photoluminescent organosilane-functionalized carbon dots as temperature probes. *Chem. Commun.* **2013**, 49, 1639–1641.
- (20) Anilkumar, P.; Cao, L.; Yu, J. J.; Tackett, K. N.; Wang, P.; Meziani, M. J.; Sun, Y. P. Crosslinked carbon dots as ultra-bright fluorescence probes. *Small* **2013**, 9, 545–551.
- (21) Jia, X. F.; Li, J.; Wang, E. K. One-pot green synthesis of optically pH-sensitive carbon dots with upconversion luminescence. *Nanoscale* **2012**, 4, 5572–5575.
- (22) Jiang, J.; He, Y.; Li, S. Y.; Cui, H. Amino acids as the source for producing carbon nanodots: microwave assisted one-step synthesis, intrinsic photoluminescence property and intense chemiluminescence enhancement. *Chem. Commun.* **2012**, 48, 9634–9636.
- (23) Li, Q.; Ohulchanskyy, T. Y.; Liu, R. L.; Koynov, K.; Wu, D. Q.; Best, A.; Kumar, R.; Bonoio, A.; Prasad, P. N. Photoluminescent carbon dots as biocompatible nanoprobe for targeting cancer cells in vitro. *J. Phys. Chem. C* **2010**, 114, 12062–12068.
- (24) Wang, J.; Wang, C. F.; Chen, S. Amphiphilic egg-derived carbon dots: rapid plasma fabrication, pyrolysis process, and multicolor printing patterns. *Angew. Chem., Int. Ed.* **2012**, 51, 9297–9301.
- (25) Liu, H. P.; Ye, T.; Mao, C. D. Fluorescent carbon nanoparticles derived from candle soot. *Angew. Chem., Int. Ed.* **2007**, 46, 6473–6475.
- (26) Sahu, S.; Behera, B.; Maiti, T. K.; Mohapatra, S. Simple one-step synthesis of highly luminescent carbon dots from orange juice: application as excellent bio-imaging agents. *Chem. Commun.* **2012**, 48, 8835–8837.